INFLUENCE OF EXPERIMENTAL THERMAL SHIFTS AND OVERCROWDING ON FECUNDITY IN WILD FEMALES OF ACARTIA TONSA OF THE BAHÍA BLANCA ESTUARY

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http://dx.doi.org/10.1590/S1679-87592014064706203

ABSTRACT

The copepod *Acartia tonsa* plays an important role in the food web of many estuaries influenced by changes due to anthropic activities, hence it is relevant to know its reproductive plasticity under stressful conditions. We examined the influence of thermal shift (6 ± 1ºC and 18 ± 1ºC) and overcrowding stress on egg production and hatching success in wild females of *A. tonsa* of the Bahía Blanca Estuary. Egg production rate (EP), hatching success during seven days (E7 %) and time taken to hatch 50 % of eggs (T 50) were registered. The high individual density used in the incubations caused a decrease in EP values at both temperatures. Nevertheless, all the females responded favorably to 18°C. EP and E7 % obtained at both temperatures were significantly different the values at 18ºC, being twice as high as those obtained at 6°C. T 50 values at 18ºC indicated a shorter developmental time in eggs from spring females, whereas the opposite effect was observed in eggs from winter females. Our results indicate that the reproductive performance is affected by short exposures to thermal shift. The pattern of egg production and hatching is also modified in accordance with the maternal history.

RESUMO

O copépode *Acartia tonsa* desempenha um papel importante na teia trófica de muitos estuários influenciados por atividade antrópica, e por esse motivo torna-se relevante conhecer sua plasticidade nos processos reprodutivos de espécies sob condições estressantes. Examinamos a influência de dois estressores, mudança térmica (6 ± 1ºC e 18 ± 1ºC) e superpopulação, na produção de ovos e sucesso da incubação em fêmeas selvagens de *A. tonsa* provenientes do estuário de Bahía Blanca. A taxa de produção de ovos (EP), o sucesso de incubação durante sete dias (E7%) e o tempo gasto para eclodir 50% dos ovos (50 T) foram registrados. A alta densidade de indivíduos usada na incubação causou diminuição nos valores de EP em ambas as temperaturas. No entanto, todas as fêmeas responderam favoravelmente a 18 °C. Os valores EP e E7% obtidos em ambas as temperaturas foram significativamente diferentes a 18°C, sendo duas vezes mais elevados do que aqueles obtidos a 6°C. Valores de T 50 em 18°C indicaram um menor tempo de desenvolvimento dos ovos das fêmeas de primavera, enquanto o efeito oposto foi observado em ovos das fêmeas de inverno. Nossos resultados indicam que o desempenho reprodutivo é afetado pela exposição curta à mudança térmica, e o padrão de produção de ovos e a incubação são também modificados de acordo com a história materna.

Descriptors: *Acartia tonsa*, Fecundity, Thermal shifts, Overcrowded incubation, Wild females.
Descritores: *Arcatia tonsa*, Fecundidade, Mudança térmica, Incubação, Fêmeas selvagens.

INTRODUCTION

Acartia tonsa (Dana), which is a euryhaline and eurythermic dominant zooplankter, especially during the warm seasons, plays an important role in the food web of several marine coastal and estuarine systems around the world (HEINLE, 1966; HOFFMEYER, 1994; MAUCHLINE, 1998; CALLIARI et al., 2009). Several studies have pointed out that the reproductive success of cultured *A. tonsa* is negatively affected by low temperatures (KATAJISTO, 2006; HANSEN et al., 2010) and overcrowding (JEPSEN et al., 2007; PECK et al., 2008; PECK; HOLSTE, 2006). Favorable temperatures (18 ± 2ºC), intermediate salinities (17 - 25) and low adult stocking densities (< 50 ind L^{-1}), are

linked to maximum egg production (20 - 40 eggs female^{-1} d^{-1}) and successful egg hatching in cultured *A. tonsa* (BAN; MINODA, 1994; CASTRO-LONGORIA, 2003; CALLIARI et al., 2006; HOLSTE; PECK, 2006; PECK; HOLSTE, 2006). Most of these studies addressing reproductive traits in *A. tonsa,* have been based either on cultured females or were performed under constant temperature and suitable feeding conditions. However, the effect of temperature shifts on copepod fecundity renders results which are more difficult to predict since wild females are naturally acclimatized to seasonal conditions of temperature and nutrition. In fact, both falls in temperature and low food availability induce a general decline in the egg production rate and the production of resting eggs in wild populations (UYE; FLEMING, 1976; KIØRBOE et al., 1985; DUTZ et al., 2004). In others estuaries, three categories or types of dormancy have been described for this copepod according to the physiological behavior: quiescent eggs, diapause eggs (GRICE; MARCUS, 1981; CASTRO-LONGORIA, 2001) and eggs with an intermediate dormancy between the two types (KATAJISTO, 2006). The quiescent eggs have the power to delay embryonic development under unfavorable conditions, a situation that lasts until favorable environmental conditions are restored (KATAJISTO, 2006). On the other hand, a maternal genetic component induces suppression of embryonic development in diapause eggs, requiring a refractory period to hatch (GRICE; MARCUS, 1981; CASTRO-LONGORIA, 2001).

The copepod *A. tonsa* is a significant key species in the inner zone of the Bahía Blanca Estuary (BBE) throughout the year. The annual pattern of its population in abundance terms shows marked seasonality and is tightly coupled with temperature (SABATINI, 1989; HOFFMEYER, 2004; HOFFMEYER et al., 2008). *Acartia tonsa* populations present their greatest abundances during the warm season, declining during the cold season when the temperature drops and becomes unfavorable in the BBE (SABATINI, 1989; HOFFMEYER, 1994; 2004). In fact, the low abundance of this calanoid during the cold season is associated with a low production of subitaneous eggs and the existence of resting eggs (SABATINI, 1989; HOFFMEYER, 2004). These resting eggs remain on the bottom sediments and contribute to the re-establishment of the population during spring (HOFFMEYER et al., 2009; BERASATEGUI et al., 2013). Little is known about the dormancy strategy of *A. tonsa* in the EBB (BERASATEGUI et al., 2013), but low egg production with no strict dormancy is to be expected during the winter. This species is adapted to the wide range of temperatures (5.1 - 26.4ºC) and salinities (17.9 - 41.3) which characterize the environmental conditions of the inner zone of the BBE (FREIJE et al., 2008). Extreme water temperatures (up to 10ºC above estuarine temperature) are frequently registered in those restricted areas of the BBE which receive hot water effluents from the cooling systems of riverside industries (HOFFMEYER et al., 2008). Moreover the impact of one outflow of overheating effluent produces the high immediate (after 24 h) mortality (40 - 50%) of *A. tonsa* copepodites, both females and males, in the affected area (HOFFMEYER et al., 2005). In fact, higher mortalities were recorded by these present authors in this impacted area during the warm season, also associated with higher population abundances. Within this context, the aim of this study was to examine the influence of thermal shift and overcrowding stress on egg production and hatching success in wild females. These results provide information on the reproductive plasticity of *A. tonsa* in the face of stressful conditions in view of the unpredictable environmental changes caused by anthropic activities.

Material and Methods

The Bahía Blanca estuary (BBE) is a mesotidal, temperate, turbid estuary, located in the southwestern Atlantic Ocean (38°44′ – 39°27′S and 61°45′ – 62°30′W) and is partially mixed with a strong tendency to be vertically homogeneous (PERILLO et al., 2001). The sampling was carried out in Puerto Cuatreros, which is located in the innermost area of this estuary. This area is relatively shallow (4 - 7 m) and highly turbid as a result of the combined effect of winds and tidal currents, which strongly enhance the resuspension of fine sediments (sand and silt-clay) (PERILLO et al., 2001). Sampling and measurements were performed on nine dates between April and October 2003 (23-Apr, 6-Jun, 19-Jun, 7-Jul, 18-Jul, 28-Jul, 8-Aug, 3-Oct, 6-Oct), covering autumn- winter (seasons of population decline) and spring (season of population recovery). On each sampling date, mesozooplankton samples were collected by means of five vertical net tows with a 200 µm mesh net and 0.30 m open-mouth. These samples were later concentrated, and transported in 5 L thermal containers to keep them at field temperature until arrival at the laboratory. Water samples were also taken using a Van Dorn bottle (2L) for the incubation medium (20 L) and for chlorophyll *a* determination. Surface temperature and salinity were recorded at the same time as the samples were collected using a HORIBA® multiparameter sonde.

In the laboratory, mesozooplankton samples were diluted with *in situ* seawater (≤ 60 µm) and transferred to 10 L plastic containers. To determine the effect of thermal shift on egg production and hatching success, wild individuals of *A. tonsa* were isolated and

exposed to 6 ± 1ºC and 18 ± 1ºC. These threshold values established the minimum and maximum abundance levels of this species in Puerto Cuatreros (SABATINI, 1989; HOFFMEYER et al., 2008). Experimental incubations, at higher densities (STOTTRUP et al., 1986) than those recorded during the warm season, were carried out without previous acclimation in order to take the influence of the female's recent history into account (GONZÁLEZ, 1974; BÅMSTEDT, 1980; STEARNS et al., 1989; TESTER; TURNER, 1990; TISELIUS et al., 1995). Ten females and one male were placed in a Petri dish containing 100 ml of filtered seawater (salinity 32 - 33.5) with ≥60 µm of *in situ* seston as food. The comparison with further findings in which overcrowding is not included will allow one to determine its degree of importance. Each incubation dish was provided with a mesh-bottom sieve of 200 µm, to avoid egg cannibalism (DAAN et al., 1988). Two-three replicates per treatment were used for each sampling date. Incubations were carried out in a temperature-controlled culture room with a fixed 12L:12D photoperiod. After 24 h incubation, adults were removed (to check the survival rate) and the eggs produced were counted under a stereomicroscope to estimate the egg production rate (EP = number of eggs per female per day). These eggs were incubated in the same Petri dishes for seven days (CASTRO-LONGORIA, 2001) to estimate the hatching success. Dishes were verified every 48 h, and the nauplii born were retained on a 60 µm sieve and then suspended and fixed in a 4 % formaldehyde seawater solution. Cumulative hatching success as a percentage, after seven days of incubation (E7 %), and the time taken to hatch 50% of the eggs produced (T 50) were estimated for each experimental temperature. EP and E7 % data were analyzed statistically using two-way unbalanced ANOVA, in which the factors were the dates and experimental temperatures. Where there was no interaction between temperatures and dates, dates were compared in pairs using the Fisher's least significant difference multiple-comparison test (LSD). When interaction was found, comparisons between dates for each temperature were also conducted using the LSD test whereas the comparisons between temperatures for each date were performed using Student's t test.

RESULT

The field environmental conditions recorded in this study during the period of the collection of the females were highly variable. The cold period (April to August) was characterized by low temperatures (6-9°C), relatively high salinities (32-33), and the occurrence of the highest chlorophyll *a* levels in early July and early August. In the spring (October), temperatures ranged between 14 and 15°C, and salinity reached minimum values (31 - 32), while chlorophyll *a* was relatively low (Fig. 1).

Incubation results showed that the egg production rate (EP) was lower at 6 ºC than at 18 ºC (n=22 total incubations) in every female analyzed (5.16 ± 2.5 eggs female^{-1} d^{-1} and 9.66 ± 4 eggs female^{-1} d^{-1}, respectively) and significant differences were detected between the two temperatures (f = 35.78, p << 0.01). However, the temporal pattern of EP was similar at both experimental temperatures, and maximum rates were registered on June 6 and July 18 (Fig. 2A). Significant differences in EP were also found between dates (f = 5.04, df8, p <0.01). No interaction between temperatures and dates was found (f = 1.09, df 8, p > 0.30). Statistical differences between dates are shown in Table 1.

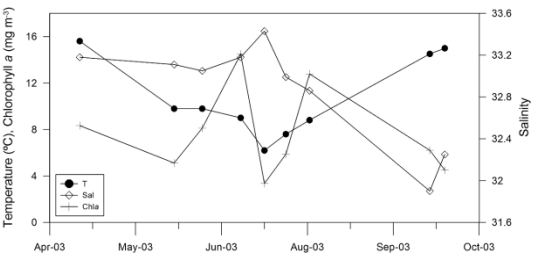

Fig. 1. Environmental *in situ* conditions during the study period.

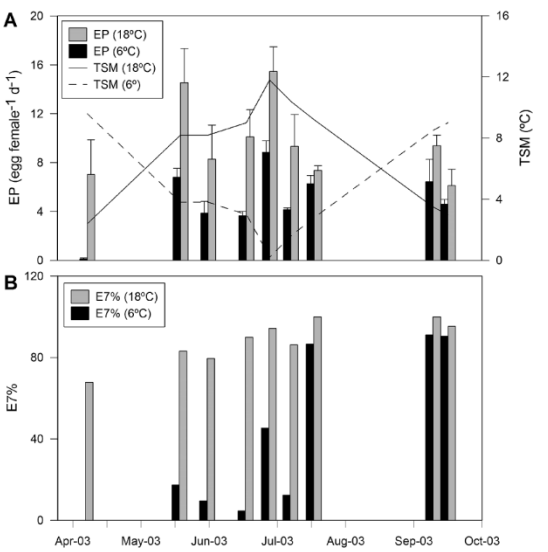

Fig. 2. Effect of thermal shifts on egg production rate (EP) and hatching success (E7 %) in field females of *Acartia tonsa*. (TSM) thermal shifts magnitude, (A) pattern of EP under 6±1ºC and 18±1ºC.Bars represent the standard error; (B) pattern of E7% (cumulative hatching success as a global estimation for all replicates after seven days of incubation).

Table 1. Results of Fisher´s LSD test for EP, E7 % and T 50% data. The letters are assigned in alphabetical order from lowest to highest average. Two dates that share at least one letter do not differ at 5%.

Fisher's LSD	EP	E7% to 6ºC	E7% to 18ºC
23-Apr	a	-	a
06-Jun	cd	a	bc
19-Jun	ab	a	ab
07-Jul	ab	a	bcd
18-Jul	d	b	bcd
28-Jul	ab	a	bcd
08-Aug	ab	c	d
03-Oct	bc	c	d
06-Oct	ab	c	cd

A higher hatching success was observed in experiments conducted at 18 °C than in those at 6ºC. Egg hatching increased at both temperatures after July 28, this fact being more evident at 6°C (Fig. 2B). E7 % showed highly significant differences between dates (f = 46.7, df 8, p <0.01) and between temperatures (f = 299.73, df 8, p <0.01). A highly significant interaction between dates and temperatures was also found (f = 23.78 df 8, p <0.01). The LSD test comparisons between dates for each experimental temperature are shown in Table 1. Highly significant differences (p <0.01) of E7 % between temperatures were found on the first five dates (Table 2). The time taken to hatch 50 % of the eggs produced (T 50) was only estimated for 18ºC, because the eggs hatching at 6 ºC did not exceed 50% during the incubation period. Mean T 50 values ranged between 2.87 and 5.45 days on the first three dates at 18ºC, and then decreased to ~ 1 to 3 days on the following four dates until reaching minimum hatching time (20 - 39 h) in October.

Table 2. Results of Student's *t*-test for.** The difference is highly significant (p <0.01), * The difference is significant (p <0.05), ns the difference is not significant (p> 0.05).

Date	Diff. (18º-6ºC)	Signif.
06-Jun		**
19-Jun	69.83	**
07-Jul	85.33	**
18-Jul	48.96	**
28-jul	73.80	**
08-Aug	13.45	*
03-Oct	8.92	ns
06-Oct	4.91	ns

DISCUSSION

The average values of EP at both experimental temperatures were lower than the EP values (i.e. 50.43–12.95 eggs female^{-1} d^{-1} during the warm season and 6.5 eggs female^{-1} d^{-1}during the winter) reported in the BBE from incubations with lower densities (< 50 ind. L^{-1}) of wild females (BERASATEGUI et al., 2013). Moreover, this negative overcrowding effect on EP was more evident in the incubations at 18 ± 1°C with spring females, since this value (7.76 eggs female^{-1} d^{-1}), was approximately 30-40% less than the EP reported by Berasategui et al. (2013). It has been reported that overcrowding in cultured calanoid is related to decreased water quality, creating stress on the individual. Moreover, cannibalism, high mortality and decrease of egg production and egg viability, were observed under these stressful conditions (PECK; HOLSTE, 2006; JEPSEN et al., 2007; CAMUS; ZENG, 2009). Thus the low egg production rate found in our study at both thermal shifts may be partly explained by the low availability of food per female and low oxygenation caused by the high experimental density. Although the overcrowding effect had an influence on egg production, significant differences were found in EP between the two experimental temperatures. EP at 18°C was twice as high as at 6°C, regardless of the natural acclimation of wild females and the magnitude of the experimental thermal shift. Moreover, the low EP values obtained under incubation at 6ºC indicated a negative effect on egg production. This is in agreement with the findings of Sabatini (1989), who suggested a decrease in the egg production rate of *A. tonsa* during the cold season as a factor contributing to the population decline in the BBE. A similar trend was observed in *A. tonsa* and species of the same genus in other estuaries worldwide (AMBLER, 1985; STEARNS et al., 1989; WHITE; ROMAN, 1992; DUTZ et al., 2004; JUNG et al., 2004). On the other hand, the incubation temperature of 18 ± 1°C benefited the fecundity of the wild females. Moreover, the highest EP was obtained at 18 ºC under incubation with the highest magnitude of thermal shift (11.8ºC). A study conducted in the BBE showed a negative influence of hot water effluents from riverside industries on the mesozooplankton community (HOFFMEYER et al., 2005). The same authors found that the thermal amplitude in the area was of up to 10 ºC, and resulted in high *A. tonsa* copepodites, female and male mortality (40-50%) during warm seasons, and lower mortality (< 40%) during cold seasons. Nonetheless, we did not find any dead females in our incubations and a quick response to thermal shift was observed after short exposure (24 h). Despite the high magnitude of the thermal shift (8 - 11.8°C) between the experimental (18ºC) and field temperatures, winter females showed enhanced fertility as compared with spring females, which faced an experimental thermal shift of 3-4°C. This behavior is probably related not only to species-specific temperature tolerance ranges but also to maternal

history. In agreement with previous studies (STEARNS et al., 1989; TESTER; TURNER 1990, TISELIUS et al., 1995), our findings suggest that the reproductive traits of *A. tonsa* are strongly associated with the nutritional history of the females. Females collected during winter (*in situ* temperature 6-10°C), showed higher mean EP at both experimental temperatures than those collected in spring with favorable *in situ* temperatures (14 - 16°C). In fact, the two higher EP values (June 6 and July 18) observed at both experimental temperatures occurred after maximum chlorophyll *a* levels, which were coupled to the winter phytoplankton bloom (POPOVICH; MARCOVECCHIO, 2008). Additionally, maximal numerical abundance of aloricate ciliates, heterotrophic dinoflagellates and rotifers has been recorded in Cuatreros Port during winter and early spring (BARRÍA DE CAO et al., 2011; PETTIGROSSO; POPOVICH, 2009). This suggests that winter phytoplankton blooms followed by abundance peaks of microzooplankton, which are the main components of the diet of *A. tonsa* (DIODATO; HOFFMEYER, 2008), modulate the EP behavior in winter females of the BBE.

Our results indicate that experimental thermal shifts also affected the hatching success. E7 % showed highly significant differences between experimental temperatures, and the lowest hatching values (5 – 45 %) were found at 6 °C in eggs from winter females. This is in agreement with studies conducted in the Baltic Sea, that reported no hatching of diapause eggs produced by females acclimated to ≤ 10ºC, incubated at low temperatures (≤ 12ºC) (CASTRO- LONGORIA, 2003; HOSTE; PECK, 2006). However, given the high hatching success of winter eggs after exposure to 18ºC in our results, they cannot be defined as diapause eggs. The female produces diapause eggs in response to cues preceding the onset of environmental adversity (GRICE; MARCUS, 1981; HAIRSTON Jr.; MUNNS Jr., 1984; BAN, 1992; BELMONTE; PATI, 2007). Moreover, these eggs must survive a refractory period during which hatching is not possible even if suitable environmental conditions are later restored (MARCUS, 1996; CHEN; MARCUS, 1997). Our results showed that egg hatching success was affected by the experimental temperature, either delaying (low temperature) or accelerating (high temperature) the emergence of the nauplii and that winter eggs seem not to require a refractory period prior to hatching. Regarding egg hatching and the influence of female history, *in situ* temperature seems to be a key factor that could be transmitted to eggs through the female line, in accordance with Tester (1985) and Hansen et al. (2010). An environmental temperature rise (>15ºC) had an evidently favorable effect on hatching success through *in situ* parental acclimation, since during spring the proportion of hatched eggs was close to 100% after incubation at both experimental temperatures (6 and 18ºC).

The results of T 50 at the experimental temperature of 18ºC evidenced a shorter development time in eggs from spring females acclimated to higher natural temperatures (>15ºC), whereas the opposite effect was observed in eggs from winter females, in agreement with Ambler (1985). The lower experimental temperature (6ºC) led to a delay in the response of the egg hatching success of eggs from winter and spring females, in accordance with Hansen *et al.* (2010), and induces the quiescence of eggs. In cooler conditions a delay in egg hatching was observed, which was more pronounced in eggs from winter-spring females, which in turn had a longer natural exposure to low temperatures.

Wild females of *A. tonsa* show a positive reproductive plasticity to water heating that does not exceed 18ºC, as occurs in the surrounding areas which receive urban wastewater or industrial discharges in the BBE. Under such conditions, this species shows a favorable response in its production and hatching of eggs, which was to be expected since a high production of viable eggs at temperatures around 18 º C had been reported for *A. tonsa* in culture (CALLIARI et al., 2006). Consequently, in closed channels impacted by urban sewage, with low tidal dynamics and high residence of individuals, the dormancy strategy of *A. tonsa*, could be masked during the unfavorable winter period. In these impacted areas, this could also generate a modification in the structuring and composition of winter mesozooplankton. Though cooling has not been reported as a result of urban activities around this estuary, the results of this study would lead one to expect some reproductive response of the species to occasional harsh winters. In 2007, the effect of a polar wave of Antarctic origin which affected the south of South America generated unusually low temperatures in the BBE (BERASATEGUI et al., 2009). During this period there was a significant decline in the abundance of *A. tonsa* and a slow recovery of the population during the spring, relative to other years (BERASATEGUI et al., 2009), which could be explained by the low production of quiescent eggs.

In conclusion our results suggest that, in the face of a heating thermal shock, the winter females were, in consequence, more favored than the spring ones. On the other hand, the thermal shift of 6 ºC influences egg production negatively and induces the quiescence of eggs, more pronounced in winter females. These findings suggest some adaptation of *A. tonsa* to adverse cooling conditions and a predictable response of resistance in the short term, which favors the survival of this species. Regardless of the response to the thermal shift and the overcrowding effect, the

parental acclimation temperature influences the time of hatching, whereas maternal feeding status mainly affects egg production.

ACKNOWLEDGEMENTS

We wish to thank the Instituto Argentino de Oceanografia and the Chemistry Laboratory staff, particularly R.O. Asteasuain and L. Kaufman, for their collaboration. We are grateful to Dr. Calliari for his comments and suggestions on this study. We also wish to thank the anonymous reviewers for their corrections, which enabled us to improve this manuscript.

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(Manuscript received 25 March 2013; revised 18 January 2014; accepted 21 March 2014)